Polar Organic Chemical Integrative Sampling (POCIS) and LC-ES/ITMS for Assessing Selected Prescription and Illicit Drugs in Treated Sewage Effluents

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T.L. Jones-Lepp[1*], D.A. Alvarez[2] , J.D. Petty[2], and J.N. Huckins[2]

[1] U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, 944 E. Harmon, Las Vegas, NV 89199, USA.
[2] U.S. Geological Survey, Columbia Environmental Research Center, 4200 New Haven Road, Columbia, MO 65201, USA.

* email: jones-lepp.tammy@epa.gov, phone: 702/798-2144, fax: 702/798-2142

Abstract

The purpose of the research presented in this paper is two-fold: (1) to demonstrate the coupling of two state-of-the-art techniques: a time-weighted polar organic chemical integrative sampler (POCIS) and micro-liquid chromatography-electrospray/ion trap mass spectrometry (μ-LC-ES/ITMS); and (2) the assessment of these methodolgies in a real-world environment - wastewater effluent - for detecting six drugs [azithromycin, fluoxetine, omeprazole, levothyroxine, methamphetamine, methylenedioxymethamphetamine (MDMA)]. In the effluent from three wastewater treatment plants (WWTP), azithromycin was detected at concentrations ranging from 15 ng/L to 66 ng/L, equivalent to the total annual release of 1 to 4 kg into the receiving waters. Detected and confirmed in the effluent from two WWTPs were two illicit drugs methamphetamine and MDMA, at 2 ng/L and 0.5 ng/L, respectively. While the ecotoxicological significance of drugs in environmental matrices, particularly water, has not been closely examined, it can only be surmised that these substances have the potential to adversely affect biota that are continuously exposed to them even at very low levels. The potential for chronic effects on human health is also unknown, but of increasing concern due to the multi use character of water, particularly in densely populated arid areas.

Introduction

In the mid-1970s, clofibric acid (the bioactive metabolite from a series of serum triglyceride-lowering drugs) was detected in a groundwater reservoir that had been replenished with treated sewage water. Subsequent investigations at wastewater treatment plants (WWTPs) revealed that treatment procedures were able to remove only 20% of this compound from the influent (Garrison et al. 1976). In similar occurrence studies, aspirin, caffeine, and nicotine also were detected in sewage sludge influent and effluents (Hignite and Azarnoff 1977). Although these findings were not pursued further at the time, improvements in detection technology over the past decade have revealed that raw sewage and treated wastewater can contain ng/L to µg/L (ppt to ppb) concentrations of numerous pharmaceuticals and personal care products (PPCPs), including not just drugs and their metabolites, but also synthetic fragrances and detergents, all of which can find their way into the natural environment after excretion or disposal by end-users (Ternes 1998, Halling-Sorensen et al. 1998, Daughton and Ternes 1999, Osemwengie and Steinberg 2001). Once present in raw sewage, these substances have the potential to enter surface or ground waters through straight-piping, from WWTP effluent, wet-weather run-off, seepage from landfills, contaminated streams and lakes, recharging of aquifers with treated sewage effluent, or drainage/deep-percolation from fields irrigated with sewage effluent. Many of these compounds are more polar than pollutants of historic concern and are not readily sorbed to the subsoil, thereby increasing their potential to enter surface or ground waters. Recently, the U.S. Geological Survey reported finding, during their national stream survey, various pharmaceuticals in waters from selected US streams (Kolpin et al. 2002).

The purpose of the research presented in this paper is two-fold: (1) to demonstrate the advantageous coupling of two state-of-the-art techniques: a time-weighted Polar Organic Chemical Integrative Sampler (POCIS) and liquid chromatography-electrospray ion trap mass spectrometry (LC-ES/ITMS); and (2) the assessment of these methodolgies in a real-world environment - wastewater effluent - for detecting six drugs [azithromycin, fluoxetine, omeprazole, levothyroxine, methamphetamine, methylenedioxymethamphetamine (MDMA)].

The four prescription drugs (omeprazole, fluoxetine, azithromycin, and levothyroxine) were chosen for their polar characteristics (lending themselves to sequestration by POCIS and analysis by LC-ES/ITMS), and the fact that they are among the most widely prescribed drugs in the US (www.rxlist.com, top 200 prescribed drugs). Few environmental occurrence data are available for illicit drugs (Daughton 2001). The two illicit drugs [MDMA (Ecstasy), and methamphetamine] were also chosen because of their polar characteristics, not having been previously reported in the peer-reviewed literature as being monitored in sewage effluent, and their prevalence and increasing usage in the US (www.dea.gov 2003). Snyder et al. (2001) reported finding several controlled substances in a lake in the Southwest, that receives sewage effluent from a large metropolitan city (> 1.6 million population). Recently at a national ground water conference, methamphetamine was reported as being present in sewage effluent from a large WWTP in California, USA (Khan and Ongerth 2003). The socio-economic significance of an efficiently reliable approach for monitoring the use of illicit substances was discussed by

Daughton (2001), who proposed the use of monitoring data to provide daily influxes of drugs and applying this data to obtain a realistic perspective on the overall magnitude and geographic extent of illicit drug usage.

While the ecotoxicological significance of drugs in environmental matrices, particularly water, has not been closely examined, it can only be surmised that these substances have the potential to adversely affect biota (i.e., bacteria, fish, amphibians, etc.) that are continuously exposed, even at very low levels. Further, the potential for chronic effects on human health is also unknown, but of increasing concern due to the increasing multi-use nature of scarce water resources. These issues are summarized by Daughton (2003).

Experimental

Drug standards. Methamphetamine and MDMA were obtained from Cerilliant Corporation (formerly Radian Corp., Round Rock, TX). Azithromycin, levothyroxine, omeprazole, and fluoxetine were obtained from U.S. Pharmacopeia (Rockville, MD)

Polar Organic Chemical Integrative Sampler (POCIS)

A more detailed explanation, laboratory recovery data, and uptake rate experiments for POCIS are reported by Alvarez et al. (2004). Brief descriptions of the POCIS procedures are presented in this paper.

Materials and Reagents. Oasis HLB was supplied by Waters Corp. (Milford, MA). Polyethersulfone (PES) membrane (47 mm diameter, 0.1 μm pore size) was provided by Pall Gelman Sciences, Inc. (Ann Arbor, MI). All solvents were Optima grade (Fisher Scientific) or equivalent.

Standard POCIS Configuration. The POCIS consists of a solid sequestration medium enclosed within a microporous membrane for the integrative sampling of hydrophilic organic chemicals (Alvarez et al. 2004; Petty et al. 2003). The sampler is an abiotic device that enables estimation of the cumulative aqueous exposure to hydrophilic organic chemicals and permits determinating of the biologically relevant time-weighted average (TWA) concentrations in water. Chemical uptake into the POCIS is rate limited by the diffusion through the aqueous boundary layer at the membrane surface. The prototype design consisted of 18 cm^2 of exposed membrane surface area and 100 mg of sequestration medium (i.e., sorbent); a surface area per mass of sorbent ratio of 180 cm^2/g is considered to be the standard configuration for all POCIS work. Larger devices were constructed for use in the field portion of the study, maintaining the ratio for surface area per mass of sorbent to conform to the standard configuration (Alvarez et al. 2004).

POCIS Extraction Procedure. Recovery of the analytes from the POCIS sorbent was achieved by transferring the sorbents into glass gravity-flow chromatography columns (1 cm i.d.) fitted with glass wool plugs and stopcocks. Methanol was used to elute the pharmaceuticals from the sorbent. The recoveries from the laboratory experiments are reported in Alvarez et al., 2004.

Extracts from the POCIS were filtered and concentrated with no other sample cleanup, to prevent the potential loss of targeted contaminants.

POCIS Uptake Rate Experiments. Calibration studies to determine POCIS sampling rates for the pharmaceuticals of interest, and the two illicit drugs, were performed. These studies involved static renewal exposures of the samplers to each analyte in glass microcosms containing 1 L of reverse osmosis (RO) water. The water was replaced with freshly fortified water (5 µg of each chemical) at regular intervals for the non-stirred and stirred exposures. For the stirred exposures, the water was refreshed daily and for the non-stirred studies, every Monday and Friday. Average temperatures of the test systems were 27 and 23 °C for the stirred and non-stirred exposures, respectively. The prescription pharmaceuticals and illicit drugs were studied by two separate calibration experiments. Renewals were continued for up to 56 days to demonstrate continuous uptake over prolonged periods by determining the analyte sampling rates at 7, 14, 28, and 56 days (Alvarez et al. 2004).

Deployment. Two canisters of POCIS, six membranes per canister, were deployed at each of the three WWTPs during the summer of 2002 for a period of 28 to 30 days. The WWTPs were located in Nevada, Utah, and South Carolina, each serving a distinctly different population (size, culturally, geographically); these sites were designated as 1, 2, and 3, respectively. The flow rates for each site during the POCIS deployments were obtained from the US EPA Permit Compliance System (PCS). PCS is a computerized management information system that contains data on National Pollutant Discharge Elimination System (NPDES) permit holding facilities (i.e., WWTPs), PCS tracks the permit, compliance, and enforcement status of NPDES facilities [http://www.epa.gov/enviro/html/pcs/pcs_query_java.html]). One site, site 1, was resampled during the winter of 2003.

Liquid Chromatography-Electrospray/Ion Trap Mass Spectrometry Materials, Reagents, and Instrumentation

Solvents and chemicals. Methanol (Burdick and Jackson, Muskegon, MI), ammonium acetate (Aldrich, St. Louis, MO), and acetic acid (Aldrich, St. Louis, MO) were used to produce the mobile phase solutions.

LC-ES/ITMS

Liquid Chromatography. The separations were performed using a Restek Allure C_{18}, 5µm particle size, 150 x 3.2 mm liquid chromatography column (Bellefonte, PA). Flow rate of 0.40 mL/min, with a 40:60 split after the column, such that 40% of the flow (160 uL/min) goes to the ES/ITMS. The injection volume on-column was 20 µL, but the volume entering the ES/ITMS would be only 8 µL, due to the 40:60 split. The gradient elution conditions were: 100% mobile phase A (hold for 1 min) to 100% mobile phase B (hold for 5 min) over a 20-min gradient. Mobile phase A: 99% water/1mM ammonium acetate/0.1% acetic acid/1% methanol; mobile phase B: 98% methanol/1mM ammonium acetate/0.1% acetic acid/2% water.

Electrospray/Ion Trap Mass Spectrometry. A ThermoQuest Finnigan LCQ™ (San Jose, CA), configured with an electrospray (ES) ion source was used to detect the pharmaceuticals. The LCQ uses an ion trap mass spectrometer (ITMS) detector that performs real-time mass analyses of liquid chromatograph (LC) eluents over a mass-to-charge ratio range of 50 to 2000. The LCQ was run in the positive ionization mode, 4.8 kV was applied to the ES needle, the heated capillary was set at 215°C, and the sheath gas was set dependent upon the optimized response of the ions of interest [these sheath gas values could range from 30 to 60, where range is arbitrarily set by the manufacturer from 25 to 100 (no units)]. The ITMS was scanned from 120 to 830 amu (full-scan mode) in 3 μscans with an ion injection of 200 ms.

Calibration, blanks, and LC/MS quantitation. For each set of LC/MS analyses a calibration curve consisting of triplicate standard solutions were analyzed. Two standards were analyzed at the beginning of each day of operation, a series of solvent blanks (until no carryover was detected), then samples (field blanks and samples), and a final standard. An external standard calibration procedure was used, the mechanics of this procedure are outlined in EPA's Solid Waste-846 manual, 8000B, section 7.4.2.1.[http://www.epa.gov/epaoswer/hazwaste/test/pdfs/8000b.pdf] For quantitation purposes a selected ion monitoring (SIM) procedure was used. This is a mass spectrometric procedure whereby only those ions of interest are monitored (see list of ions in table 1), and the areas under the SIM ion chromatogram peaks are quantitated using a manual quantitation procedure as provided by the software on the LCQ. The concentrations of the target analytes were determined using the POCIS uptake rates and applying those values obtained to concentrations quantitated in the extract by LC/MS, the number of POCIS membranes used, the flow rate of the WWTP during the time of sampling [as reported in the PCS], and any dilution or concentration factors that were necessary.

MS/MS Experiments. The LCQ can be used to perform collision induced dissociation (CID) experiments (MS^n). The precursor ion of interest is isolated in the ion trap, voltages are applied to the trapped ions inducing collisions and subsequently product ions (ions that are produced from the precursor ion). The collision energy (CE) is related to the precursor ion and the amplitude of the resonance excitation radio frequency (RF) voltage. CE is the percent of a maximum voltage used to accelerate ions into collisions. Each ion trap has unique slope and intercept of the amplitude of the RF, but each manufacturer ensures that these values are normalized to a percent of the amplitude voltage, thereby guaranteeing (all other conditions the same) that MS^n spectra are reproducible from instrument to instrument. For the ion trap used in this research the slope is 0.001126 V/μ and intercept is 0.4 V. The collision energies (CE) depend on the precursor ion selected, usually the most abundant ion, and the amount of fragmentation desired for confirmation; in these studies they ranged from 15 to 30% (see Table 1).

Results and Discussion

Most conventional environmental pollutant screening techniques for water matrices use grab sampling coupled with solid phase extraction (SPE) or liquid-liquid extraction (LLE). However, there are limitations to these techniques. Grab samples give an incomplete picture of overall concentrations of pollutants. For example, a study by Williams et al. (2003) showed that daily grab samples of waters taken from a river had a wide variance in daily estrone concentrations. Another drawback to SPE and LLE is their limited capacity, sample volume sizes are usually 1- to 2-L, thereby limiting the detection limit. Some of the strengths of the POCIS are its capacity to handle large volumes of water [millions of gallons/day (mgd)] over a period of several days or weeks,thereby giving TWA concentrations, and its ability to detect episodic changes in environmental contaminant concentrations, which are often missed with conventional grab samples. POCIS is capable of concentrating very polar analytes (such as the pharmaceuticals of interest in this study) and the membrane design allows for the selective sampling of the residues from the dissolved (bioavailable) phase, allowing POCIS to be deployed under nearly all environmental conditions regardless of water quality. For the purpose of this pilot study it was decided to use the POCIS sampling technique, for a 30-day deployment at three geographically distinct and diverse population sites: Nevada (site 1), Utah (site 2), and South Carolina (site 3).

Two deployment canisters, each containing six POCIS, were placed *in situ* at each site during the summer of 2002. The samplers were placed such that they would be immersed in the effluent stream inside the WWTP, just before discharge into local receiving waters. During the sampling period at site 1 this WWTP experienced difficulties in flow regulation during the deployment period (according to the plant manager), such that the samplers were sometimes out of the water over the 30-day sampling period. Therefore, it was decided to re-deploy the samplers for another 30-days during the winter of 2003, just downstream (in the receiving waters) from the WWTP effluent discharge at site 1, ensuring that the POCIS would be submerged for the entire deployment period (hereafter referred to as site 1-II). After the 30-day deployment, the samplers were retrieved and sent back to USGS-CERC for extraction. The six POCIS from each canister were combined, thereby generating two extracts (six POCIS composite each) per site. These extracts were sent to EPA-Las Vegas and analyzed by LC-ES/ITMS for the six targeted drugs. Listed in Table 1 are the molecular weights, the most abundant electrospray ions, the MS/MS ions (precursor and product),and the LC-ES/ITMS limits-of-detection (LODs), for these six drugs. Listed in Table 2 are the targeted drugs and their concentrations found at the three sites, including the repeated sampling at site 1.

At all three sampling sites azithromycin was detected. Azithromycin is one of the most widely prescribed antibiotics in the US. Under full-scan mode, two ions are present for azithromycin: 749.4 m/z (100% relative intensity), corresponding to the $(M+H)^+$ ion, and 771.4 m/z (10% relative intensity to 749.4 m/z), corresponding to $(M+Na)^+$. For confirmation, ion 749.4 m/z was chosen for CID experiments. At 25% CE, the formation of the product ion 591.4 m/z [loss of one ring structure, $(M+H-C_8H_{16}O_2N)^+$] is induced; the precursor ion, 749.4 m/z, is still present, but at a much lower intensity (see figure 1). This same CID experiment was performed on the sample extract. The resultant product ion formation (749.4 m/z —> 591.4 m/z), along with a similar retention time as the standard, confirmed the initial identification of azithromycin (see

figure 2). The amount of azithromycin detected at site 1 ranged from 15 ng/L (summer 2002) to 66 ng/L (winter 2003). This is equivalent to the total annual release of 1 to 4 kg into the receiving waters. The other two sites had concentrations ranging from 17 to 56 ng/L, equivalent to the annual release of 0.4 to 2 kg of azithromycin.

Many effluents go directly into receiving streams that are subsequently used downstream as drinking water, not to mention the myriads of bacterial organisms being continually bathed in this antibiotic laden effluent. The occurrence of antibiotic-resistant bacteria in waters receiving wastewater effluents is being reported with increasing frequency (Andersen 1993, Guardabassi et al. 1998, Iwane et al. 2001, Ozkanca et al.1997, Schwartz et al. 2003). Although this resistance probably originates from gene-transfer from the shedding of bacteria that have been exposed to therapeutic concentrations, the environmental significance of antibiotic concentrations orders of magnitude lower cannot be discounted (Daughton 2002). As stated in Schwartz et al. (2003), the antibiotic concentrations found in wastewater are probably lower than that necessary to inhibit the growth of resistant bacteria, but they are at levels likely to affect susceptible bacteria and determine selection of more resistant bacteria as shown by their toxicity tests.

Detected in the extract from site 1 (both summer and winter sampling) was the illicit drug methamphetamine, ranging from 0.8 to 1.3 ng/L (winter and summer, respectively). This is equivalent to the total annual release of 0.05 to 0.11 kg into the receiving waters. In full-scan positive ionization mode, the most abundant ion detected for methamphetamine is 150 m/z, the $(M+H)^+$ ion. In the sample extracts, the retention time of methamphetamine had shifted due to the large surfactant peaks present. Therefore, to confirm that the 150 m/z ion was methamphetamine, CID experiments were performed. At 20% CE, the formation of the product ion 118.9 m/z [$(M+H-CH_3NH)^+$] is induced; the precursor ion,150 m/z, is still present (see Figure 3). These same ions were confirmed in the sample extracts (see Figure 4). For final confirmation a known amount of methamphetamine was spiked into the sample extract. The spiked methamphetamine, 150 m/z ion, eluted at the same retention time as the original 150 m/z ion in the unspiked sample extract. It may not be surprising to find methamphetamine in these sample extracts as a recent report from the Drug Enforcement Administration (DEA) concludes that methamphetamine is a problem in the metropolitan area served by site 1 (www.dea.gov/pubs/states.html, 2003). Also it is known that approximately 62% of an oral dose of methamphetamine is eliminated in the urine within the first 24 hours with about one-third as intact drug and the remainder as metabolites (www.rxlist.com/cgi/generic2/methamphetamine_cp.htm 2003)].

The illicit drug MDMA was detected in the extract from site 3, at a concentration of 0.5 ng/L. MDMA, like methamphetamine, is mostly excreted as the parent compound, 72% within 72 hrs. (Helmlin H-J, et al.1996). This is equivalent to the total annual release of 0.02 kg into the receiving waters. Under full-scan mode, the most abundant ion detected for MDMA is 194.0 m/z, the $(M+H)^+$ ion. In the sample extract, the retention time of MDMA had also shifted, due to the large surfactant peaks. CID experiments were performed to confirm the presence of MDMA. At 20% CE, the formation of the product ion 163.0 m/z [$(M+H-CH_3NH_2)^+$] is induced; the precursor

ion, 194.0 m/z, is also present (see Figure 5), these same ions were confirmed in the sample extract (see Figure 6). For final confirmation a known amount of MDMA was spiked into the sample extract. The spiked MDMA, 194 m/z ion, eluted at the same retention time as the original 194 m/z ion in the unspiked sample extract. According to the DEA, MDMA is known as a "club" drug and is popular with those who frequent the rave scene [***rave*** is a popular term for "An underground party featuring a distinctive style of music, dress, dance and visual effects in combination with open sexual behavior and psychedelic chemicals such as ecstasy (www.urbandictionary.com 2003)]. A recent report from the DEA concludes that MDMA is a growing problem in the metropolitan area served by site 3 (www.dea.gov/pubs/states.html, 2003).

The LC-ES/ITMS analysis of the POCIS extracts also included a screening for non-target compounds. Many types of surfactants were detected at all three sites, for example, nonylphenol polyethoxylates (NPEOs), alcohol polyethoxylates (APEs), and as yet unidentified series of surfactants [possibly a series of polyethylene glycolates (PEGs), but unconfirmed at this time]. The spectra obtained by LC-ES/ITMS, of the three types of surfactants observed, indicated a homologous series of ions 44 amu apart, which can be attributed to the ethoxylate units [-O-CH2-CH2-] of NPEOs, APEs, and PEGs. The appearance of these types of surfactants in wastewaters was not unexpected as they are present in a multitude of consumer products (i.e., detergents, soaps, shampoos) and have been reported in the literature for almost 20 years (Ahel and Giger 1985) and more recently by Petrovic and Barceló (2001). Recently Cohen et al. (2001) have reported in the literature an extensive table of the LC-ES/MS ammoniated masses of the alcohol- and alkyl- polyethoxylates. The NPEO spectra (spectrum in figure 7 is an average of the ion current under the shaded area), and the APE spectra (spectrum not shown), obtained in our studies accord exactly with Cohen et al. (2001) table of expected LC-ES/MS ammoniated ions.

These findings represent but a minuscule fraction of drugs (prescribed and illicit) that might occur in WWTP effluents. We believe that this pilot study shows for the first time the practicality of using a TWA sampler in order to better understand the broader presence of drugs being released into the environment. We believe this data represents the first time illicit drugs have been confirmed and quantified in wastewater effluents, in the peer-reviewed literature. These findings may spur socio-economic researchers to examine using environmental monitoring to better understand a community's drug habits in order to better target anti-drug campaigns (Daughton 2001). Further, monitoring illicit drug use, employing the combination of integrative sampling and LC/MS could be applied to a wide variety of situations. One result of finding azithromycin at all the WWTP sites might lead to future temporal studies of antibiotic release from various WWTPs, perhaps prompting improved engineering practices to reduce antibiotic load into receiving waters.

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Table 1. CAS numbers, molecular weights, ES ions, and LODs for Six Drugs by LC-ES/ITMS.

Analyte (CAS number)	Molecular weight (daltons)	ES/ITMS Ions generated (% relative abundance > 5%)	ES-MS/MS ions generated (% relative abundance > 5%), CE%	LOD[a]
Azithromycin (83905-01-5)	748.4	749.4 $(M+H)^+$ [100] 771.4 $(M+Na)^+$ [10]	591.4 $(M+H-C_8H_{16}O_2N)^+$ [100], 25 749.4 $(M+H)^+$ [8]	4 ng
Fluoxetine (59333-67-4)	309.3	310.3 $(M+H)^+$ [100]	310.3 $(M+H)^+$ [100], 20 148 $(M-C_7H_4F_3O+H)^+$ [66]	20 ng
Levothyroxine sodium salt (51-48-9)	798.9	777.9 $(M-Na+2H)^+$	731.9 $(M-CHO_2)^+$ [100], 20 760.9 $(M-OH+H)^+$ [10] 633.9 $(M-OH-I+H)^+$ [5]	720 pg
Omeprazole (73590-58-6)	345.4	342.4[b] $(M+H)^+$	342.4[b] (M+H) [100], 35 310.4 (M-CH3O+H) [30]	1 ng
Methamphetamine (537-46-2)	149	150 $(M+H)^+$ [100] 118.9 $(M+H-CH_3NH_2)^+$ [5]	150 $(M+H)^+$ (100), 20 118.9 $(M+H-CH_3NH_2)^+$ [15]	190 pg
MDMA (69610-10-2)	193	194 $(M+H)^+$ [100]	194.0 $(M+H)^+$ [100], 20 163.0 $[M-CH_3NH_2+H]^+$ [65]	250 pg

[a] On-column detection limit, as determined by ref MacDougall et al., 1980. [b] where via benzylic cleavage and cyclization, $(M+H)^+ = (C_{17}H_{16}N_3O_3S)$

Table 2. Concentrations (ng/L) of six drugs from three Wastewater Effluents and one stream using POCIS and μ-LC-ES/ITMS.

Site (flow) season sampling dates Analyte	1 (60 mgd)[a] summer (June18-July 18, 2002) ng/L	1-II (41 mgd)[a] winter (Jan. 9-Feb. 6, 2003) ng/L	2 (17.5 mgd)[a] summer (June 19-July 19,2002) ng/L	3 (30 mgd)[a] summer (July 1-30, 2002) ng/L
Fluoxetine	nd	nd	nd	nd
Omeprazole	nd	nd	nd	nd
Azithromycin	15[b, c] (± 2)	66[b, c] (± 14)	17[b, c](± 0.2)	56[b, c] (± 5)
Levothyroxine	nd	nd	nd	nd
Methamphetamine[d]	1.3[c]	0.8[b, c] (±0.1)	nd	nd
MDMA[d]	nd	nd	nd	0.5[c]

[a] As reported in the Permit Compliance System (PCS). PCS is a computerized management information system that contains data on National Pollutant Discharge Elimination System (NPDES) permit holding facilities. PCS tracks the permit, compliance, and enforcement status of NPDES facilities [http://www.epa.gov/enviro/html/pcs/pcs_query_java.html] [b] Average value from two canisters. [c] Confirmed by ms/ms. [d] For methamphetamine and MDMA the ms/ms ions (118.9m/z and 163 m/z, respectively) were used for quantitation purposes, due to interferences.

Figures

1. Standard ion chromatogram and CID mass spectra of azithromycin
2. Sample (site 1-II) ion chromatogram of CID mass spectra of azithromycin
3. Standard ion chromatogram and CID mass spectra of methamphetamine
4. Sample (site 1-II) ion chromatogram and CID mass spectra of methamphetamine
5. Standard ion chromatogram and CID mass spectra of MDMA
6. Sample (site 3) ion chromatogram and CID mass spectra of MDMA
7. Total ion chromatogram and spectral plot of nonylphenol ethoxylate (site 3).

Figure 1

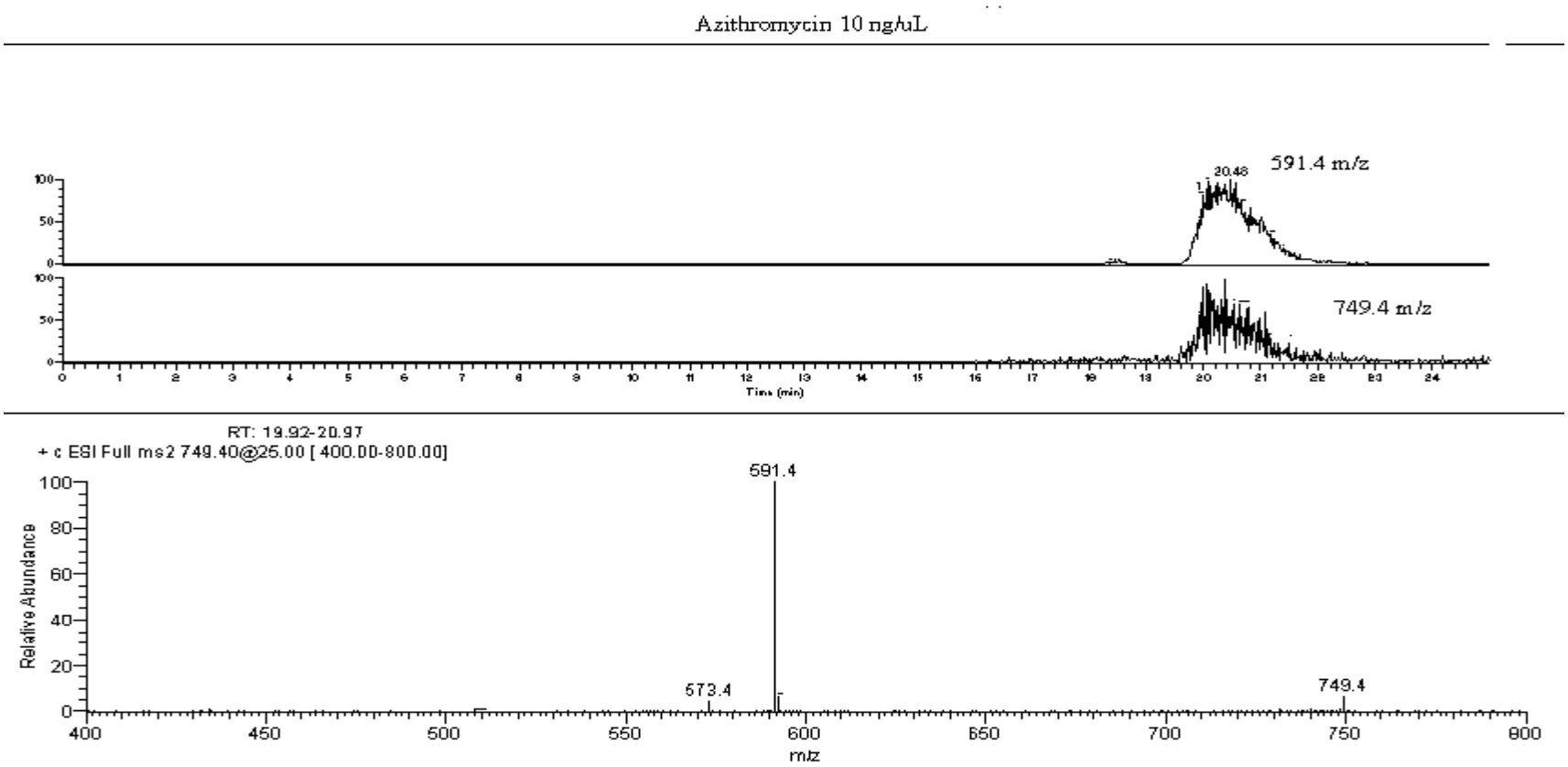

Azithromycin 10 ng/uL
591.4 m/z
20.48
749.4 m/z
Time (min)
RT: 19.92-20.97
+ c ESI Full ms2 749.40@25.00 [400.00-800.00]
591.4
573.4
749.4
Relative Abundance
m/z

Figure 2.

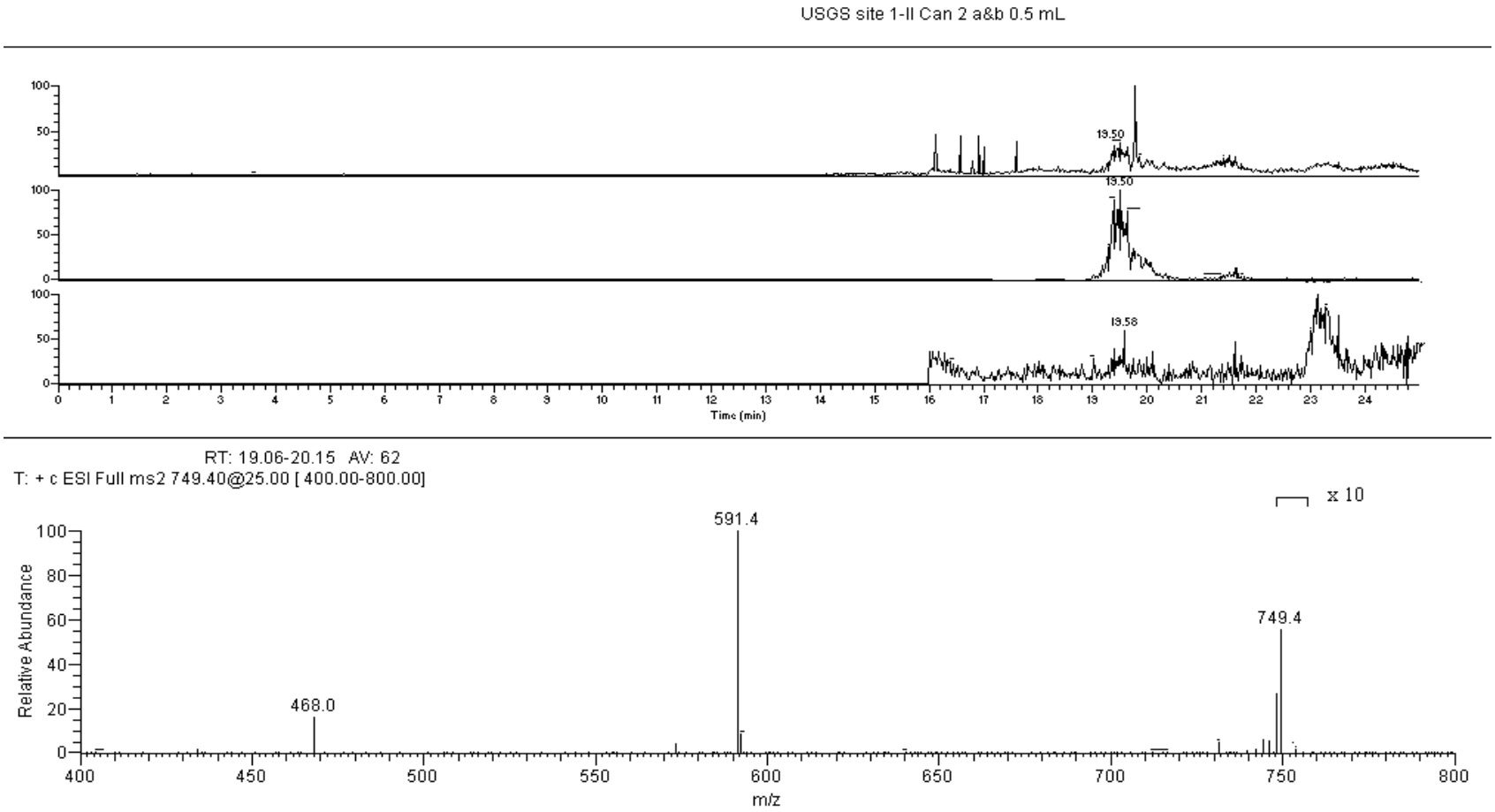

USGS site 1-II Can 2 a&b 0.5 mL
13.50
13.50
13.58
Time (min)
RT: 19.06-20.15 AV: 62
T: + c ESI Full ms2 749.40@25.00 [400.00-800.00]
x 10
591.4
749.4
468.0
Relative Abundance
m/z

Figure 3

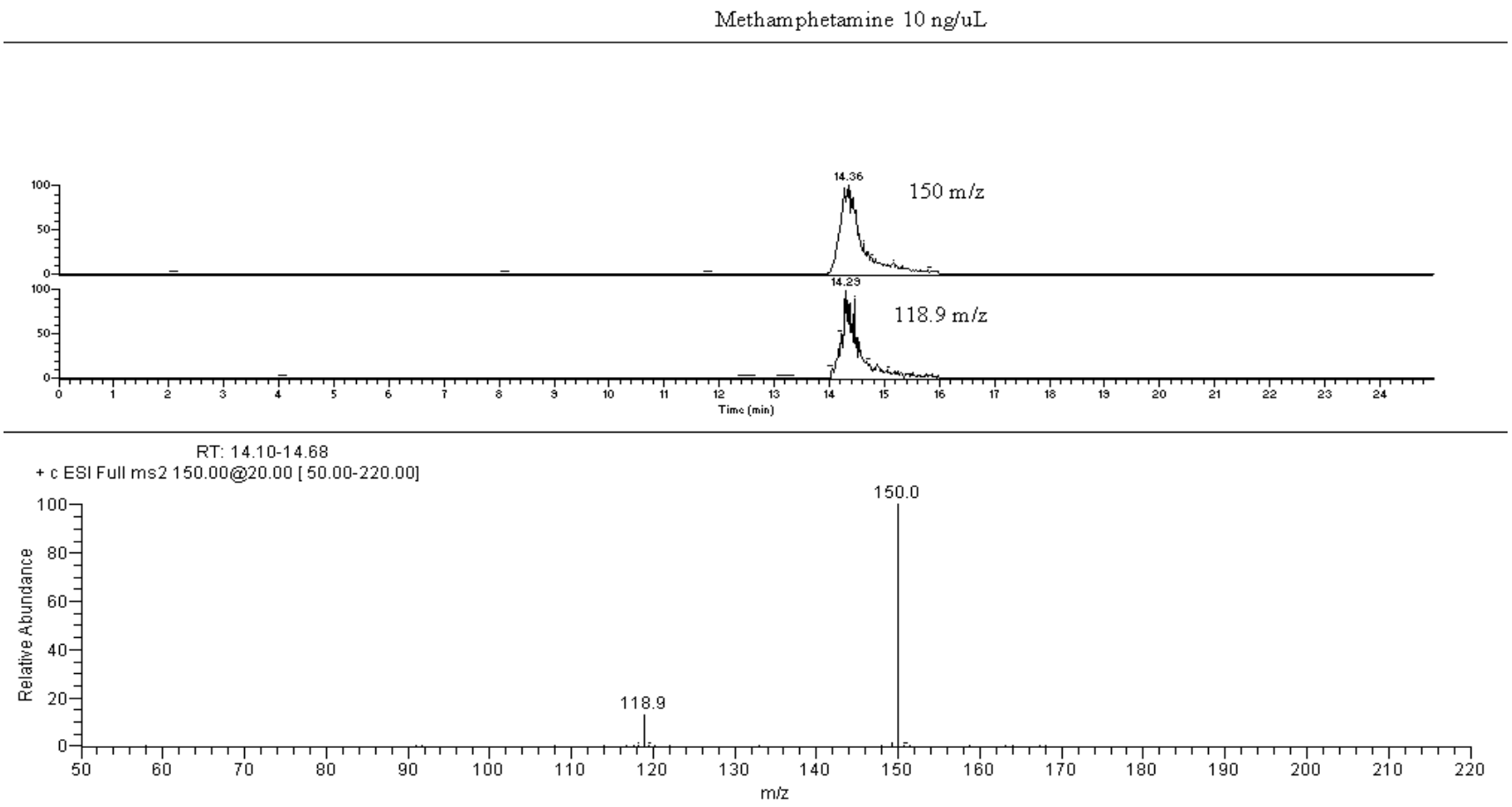

Methamphetamine 10 ng/uL
14.36
150 m/z
14.29
118.9 m/z
Time (min)
RT: 14.10-14.68
+ c ESI Full ms2 150.00@20.00 [50.00-220.00]
150.0
118.9
Relative Abundance
m/z

Figure 4

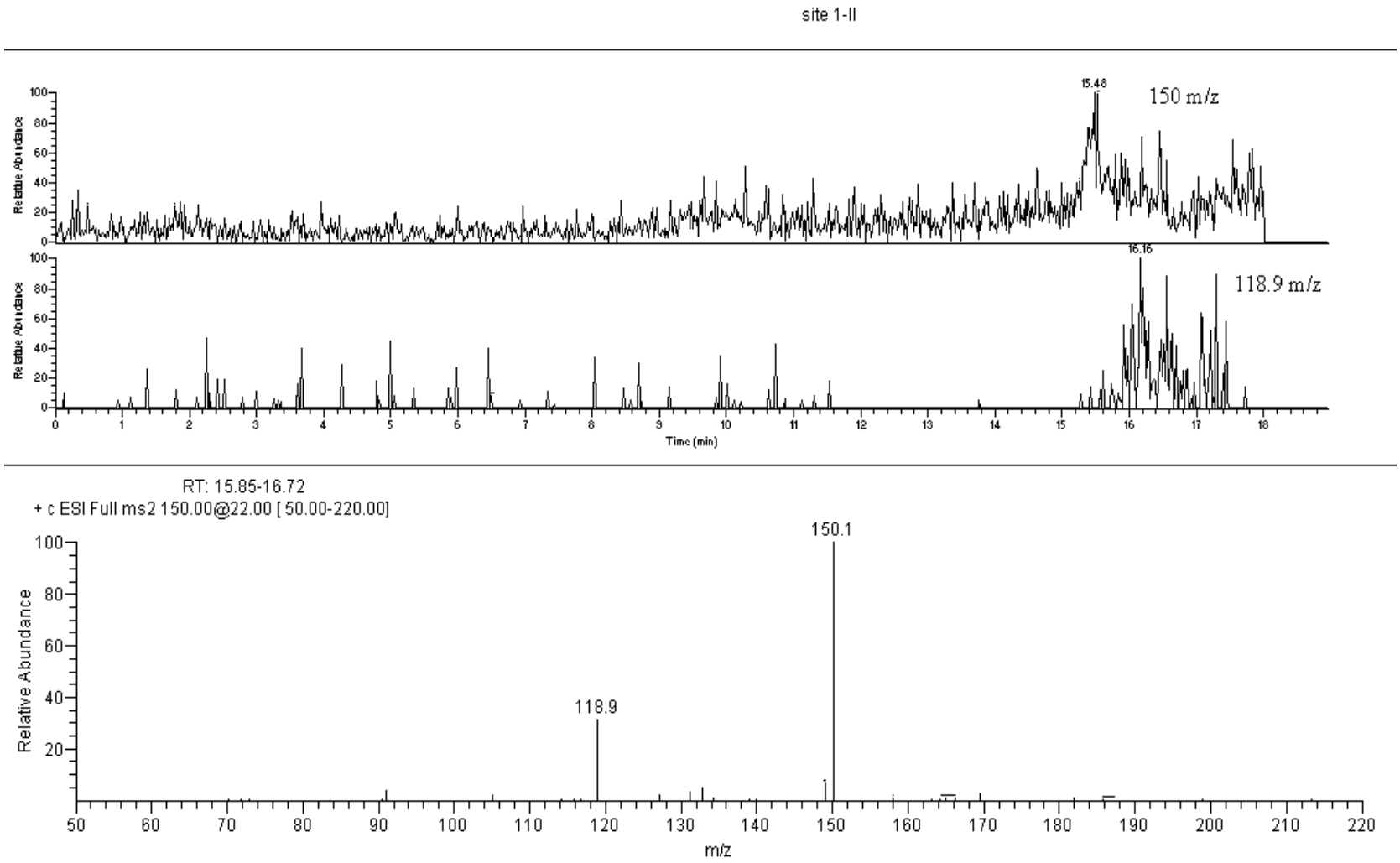

site 1-II
15.48
150 m/z
16.16
118.9 m/z
Relative Abundance
Time (min)
RT: 15.85-16.72
+ c ESI Full ms2 150.00@22.00 [50.00-220.00]
150.1
118.9
Relative Abundance
m/z

Figure 5

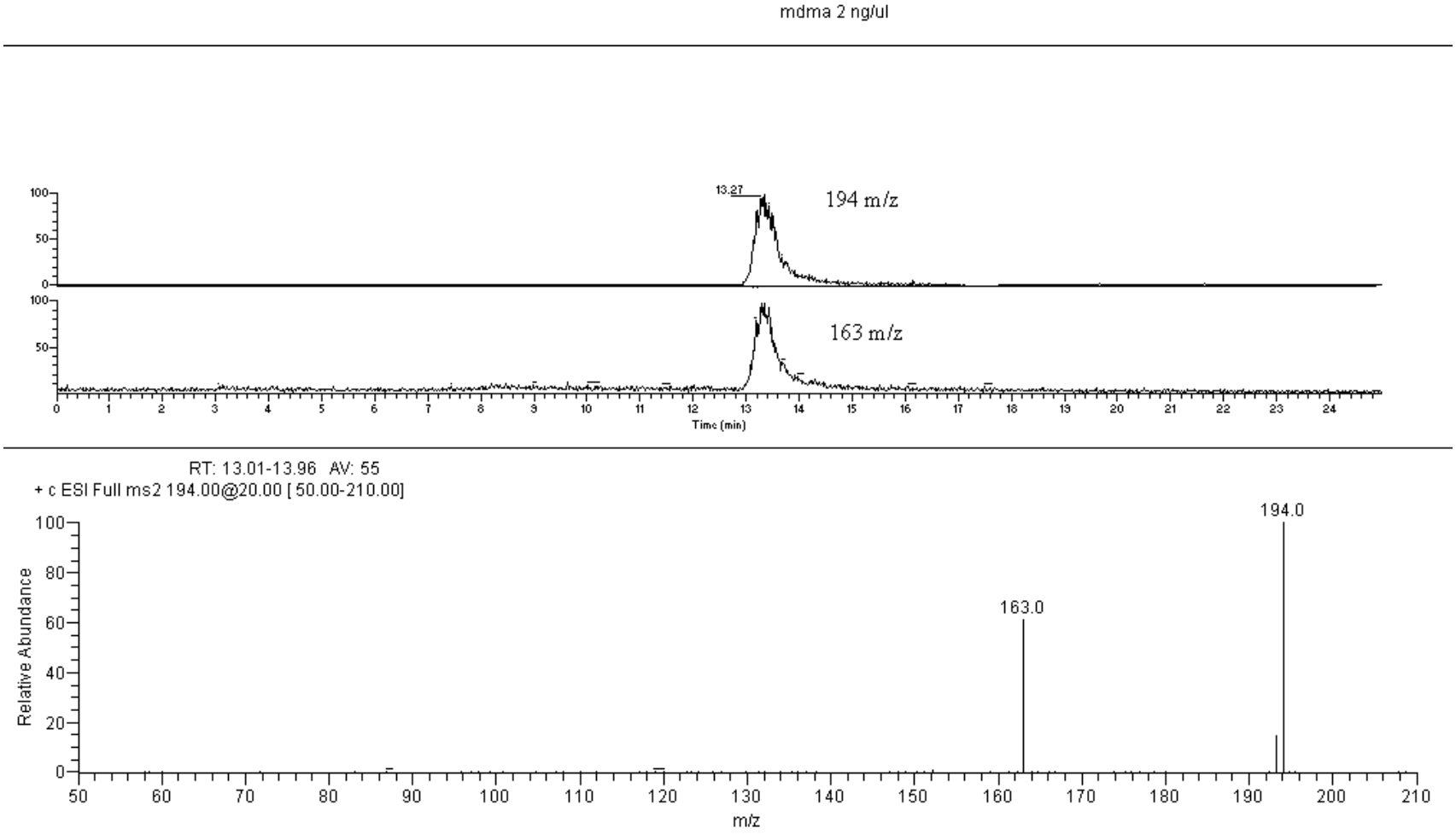
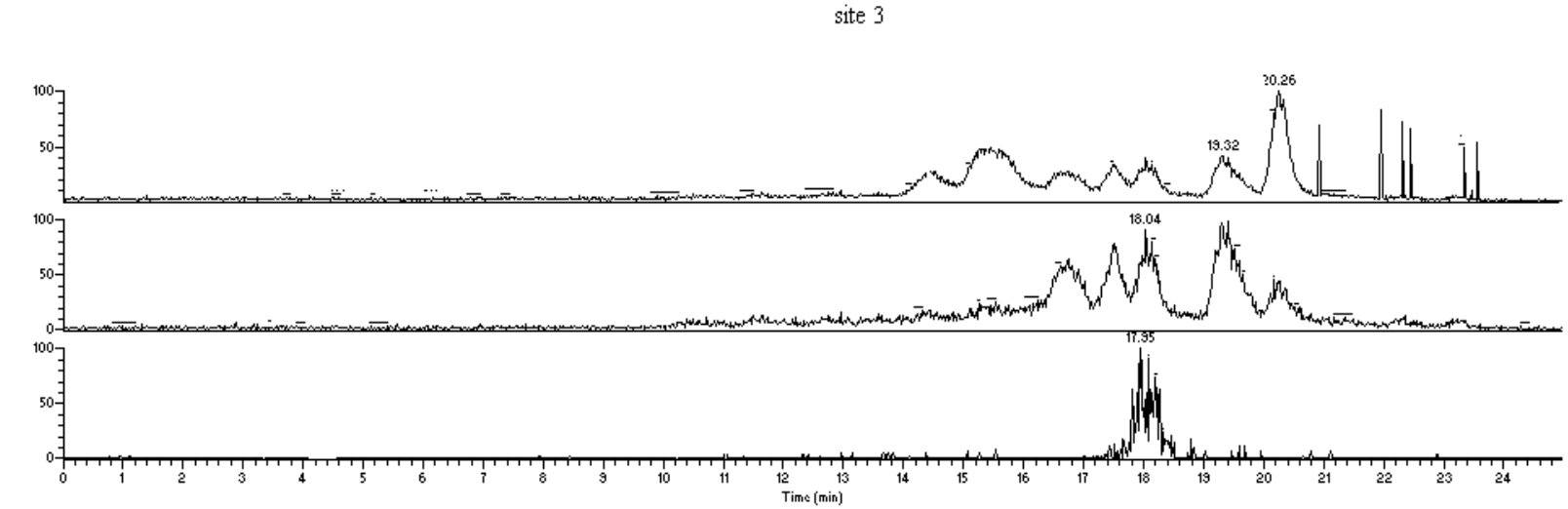

mdma 2 ng/ul
13.27
194 m/z
163 m/z
Time (min)
RT: 13.01-13.96 AV: 55
+ c ESI Full ms2 194.00@20.00 [50.00-210.00]
194.0
163.0
Relative Abundance
m/z

Figure 6


site 3
20.26
19.32
18.04
17.95
Time (min)

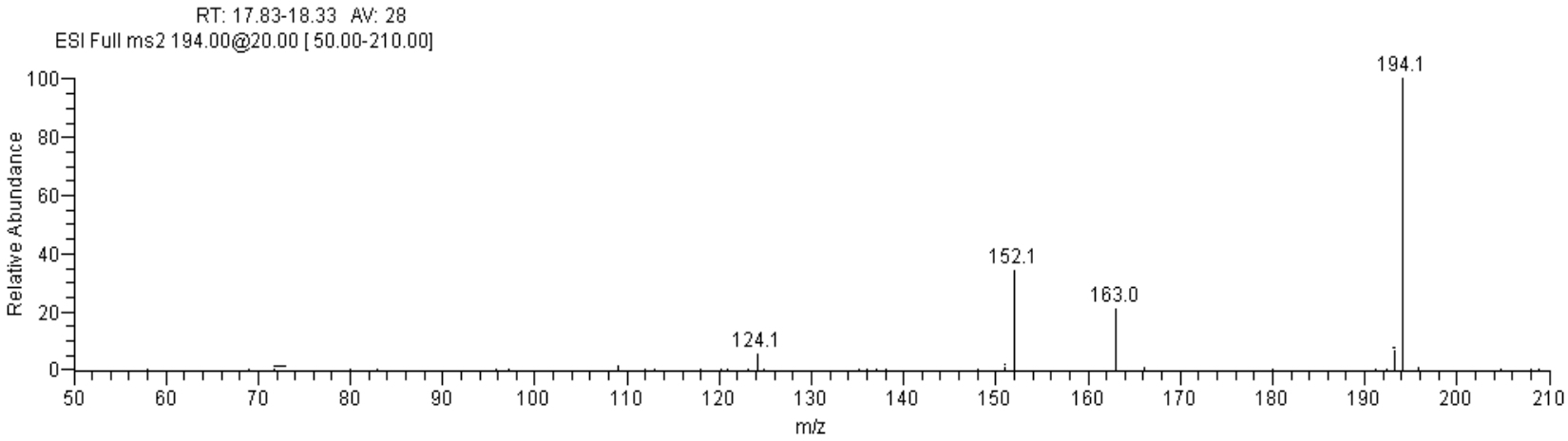
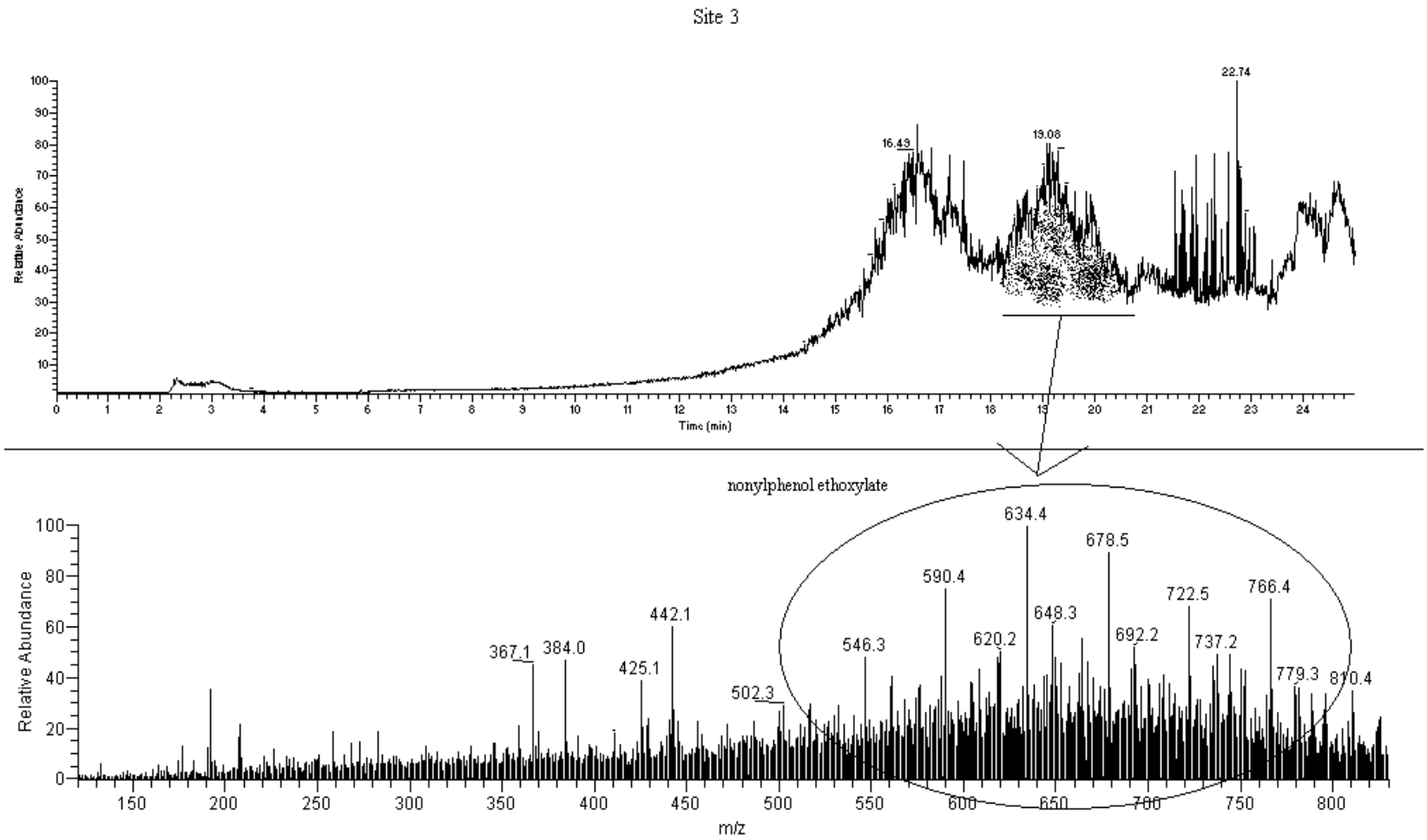

RT: 17.83-18.33 AV: 28
ESI Full ms2 194.00@20.00 [50.00-210.00]
Relative Abundance
m/z
194.1
152.1
163.0
124.1

Figure 7


Site 3
Relative Abundance
Time (min)
16.43
19.08
22.74
nonylphenol ethoxylate
Relative Abundance
m/z
367.1
384.0
425.1
442.1
502.3
546.3
590.4
620.2
634.4
648.3
678.5
692.2
722.5
737.2
766.4
779.3
810.4